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Beta-Lactamase Inhibitory Effect Of Some Medicinal Plants.

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ABSTRACT

Acetone extracts of ten medicinal plants at various concentrations (100 -500 µg ml^{-1}) were used to estimate their inhibitory effect on β-lactamase activity by the in vitroiodometry method (spectrophotometrically). The results exhibited thattheβ-lactamase activity of both S. sciuri and K. pneumoniae was inhibited by acetone extracts of ten medicinal plants. The IC_{50} for acetone extracts of ten medicinal plants it was calculated. The highest IC_{50}forS. sciuriβ-lactamase were 210, 216 and 230 forS. terebinthifolius, E. camaldulensis and C. roseus,respectively. However, their values forK. pneumoniae β-lactamase were 232, 251 and 280 forS. terebinthifolius, E. camaldulensis and C. roseus, respectively. The Ki values for S. sciuri β-lactamase inhibition were 211, 330 and 381 respectively for plant extracts of C. roseus, E. camaldulensis and S. terebinthifolius. Also, the Ki for K. pneumonia β-lactamase inhibition were 77, 221 and 374, respectively for C. roseus, E. camaldulensis and S. terebinthifolius.

Keywords: β-lactamase, inhibitory effect**,** Staphylococcus sciuri, Klebsiella pneumoniae, medicinal plants.

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INTRODUCTION

Beta-lactamase (EC 3.5.2.6) is an enzyme formed by medically important Gram-positive and Gram-negative bacteria, and is in charge for their resistance to β-lactam antibiotics like penicillins, monobactams, cephalosporins and carbapenems, though carbapenems are comparatively resistant to β-lactamase [1].

For more than 50 years, therapeutic control of β-lactamase-producing bacteria has been amain medical problem. Patients with resistant bacterial infections are predominating much more likely to die, In addition, survivors have significantly long-term hospital stays, delayed recovery, and long-term inability [2].

Improvement of a β-lactamase inhibitor, which conserves the β-lactam antibiotic from the effect of the β-lactamase has given scientists a novel tactic to controlling resistant microbes [3]and has been widely used in the therapy of human bacterial infections. However, the present marketed β-lactamase inhibitors (sulbactam, tazobactam and clavulanate) are not effective against all β-lactamases [4]and someβ-lactamases were resistant to clavulanic acid. So there is a necessity fornovel β-lactamase inhibitors to be joined with β-lactam antibiotics to combat against the resistant bacteria [5, 6].

Recently, there need been developing interest in alternative therapies and the therapeutic utilization of natural products, particularly those obtain from plants [7].

Medicinal plant which contain of antimicrobial compounds have considerable therapeutic and prophylactic effective as they have least side effects as contrasted with manufactured drugs and furthermore minimal possibility of development of resistance [8].There is additionally a probability that plants and herbs go about as inhibitors of β-lactamase enzymes. In addition, the plant extracts can have synergistic activity with an antibiotic.Lately, one from the strategies to lessen the resistance of antibiotics is using β-lactamase inhibitors from plant source[9].

The aim of the present work to assay the efficiency of ten medicinal plants growing in Egyptian environment as β-lactamase inhibitors.

MATERIAL AND METHODS

Beta-lactamase sample

The two bacterial isolates (Staphylococcus sciuri and Klebsiella pneumoniae) used in the present investigation were obtained from laboratory of clinical microbiology of the Faculty of Medicine at Mansoura University,Dakahlia Governorate, Egypt from clinical specimens of patients, and were screened for β-lactamase production by phenotypic methods (iodometric method and acidimetric method) according to Livermore and Brown [10], and identified in microbial laboratory of Mansoura University hospital for children by using Microscan Walk A way system (2013 Siemens Healthcare Diagnostics Inc., UK).

Beta-lactamase was isolated from both clinical isolates. The isolation was carried out according to Hedberget al. [11], and the purification of the crude enzyme extracts was carried out at 4ºC according to Ranadeet al. [12]by several steps included precipitation with ammonium sulphate at 80% saturation, DEAE-Cellulose and gel filtration on Sephadex G-200 column.

Medicinal plants samples collection

The plant materials used in this study included theflowers, leaves or pods of 10 medicinal plant species (Azadirachtaindica, Carica papaya, Catharanthusroseus, Ceratoniasilique, Eucalyptus camaldulensis, Ficussy comorus, Moringaoleifera, Ocimumbasilicum, Schinusterebinthifolius and Withania somnifera) were collected from the vicinity of the research farm of Mansoura University, Dakahlia Governorate, Egypt.

Samples were identified by a botanist (a taxonomist of medicinal plants and traditional medicine) at the Botany Department / Faculty of Sciences.The above-mentioned plants were chosen and used in this study as they have antibacterial, antioxidant, antidiabetic, anti-inflammatory and other activities.

The plants material had been washed under running tap water, and discharged under the shade to prevent potential damage to phytochemical components. They have been stocked in air-tight containers at room temperature tillneeded for usage [13].

Preparation of plant extracts

The acetone extracts were prepared according to Djeussiet al. [14]with simple modification, by impregnating 150g each of the grind dry plant materials in500 mlofsolventsfor48hr at room temperature with shaking. The extracts were filtrated via cotton wool and then through What man No.1filterpaper to remove the plant remains. The filtered extracts were intensified via using arotary evaporator with the water bath regulates at40˚C to obtain the crude extracts.Thepercentage yields of extracts between 8–20% w/w. The crude extracts were preserved at 4˚C in sterilecontainers unto more uses.

Sterility testing of the extracts

The acetone extracts of the plants were examined for sterility using the method of Sherwaniet al. [15]. One ml of every extracts was place on test tube including 5ml of sterilized nutrient broth. They were then incubated at 37˚C for 24 hrs. After incubation, the tubes were clear showing the absence of the contamination which would have caused a turbid appearance in the tubes.

Iodometry assay of inhibitory effect of plant extracts (spectrophotometrically)

Acetone extracts at various concentrations (100 -500 µg ml^{-1}) were used to estimate theirinhibitory effect on β-lactamase activity by the in vitroiodometry method. This method based on the reduction of iodine via the hydrolyzed substrate which can be specified spectro photo metrically. This assay is considerably used to measure the β-lactamase activity of several substrates. To comprehension this principle, a modified method was designed todetermine the β-lactamase inhibitory activity of the plant extracts.

A modified iodometry method [8]was used to carry out this experiment with some exceptions like using of $HgCl_2$ as the standard enzyme inhibitor during the investigation. Likened to penicillin G, the same amount of $HgCl_2$ was dissolved in phosphate buffer and used as the positive control. As the negative control; sterile distilled water was used.

RESULTS

The results showed thatthe β-lactamase activity of both S. sciuri andK. pneumoniae was inhibited by acetone extracts of ten medicinal plants mentioned above. It was observed thatthe remaining activity started to reduce from 100% in the control value gradually until it declined and reached to values vary depending on the type of plant. At both cases and as general phenomenon the reduction in the enzyme activity was dependent on the concentration (Fig. 1, 2, 3, 4, 5, 6, 7, 8, 9, 10).

Calculating the IC_{50} for acetone extracts of ten medicinal plants it was summarized in Table 1for the two bacteria S. sciuri and K. pneumoniae β-lactamase.

For S. sciuriβ-lactamase the highest IC_{50} were 210, 216 and 230 for S. terebinthifolius, E. camaldulensis and C. roseus,respectively. However, for K. pneumoniae β-lactamase their values were 232, 251 and 280 for S. terebinthifolius, E. camaldulensis and C. roseus, respectively.

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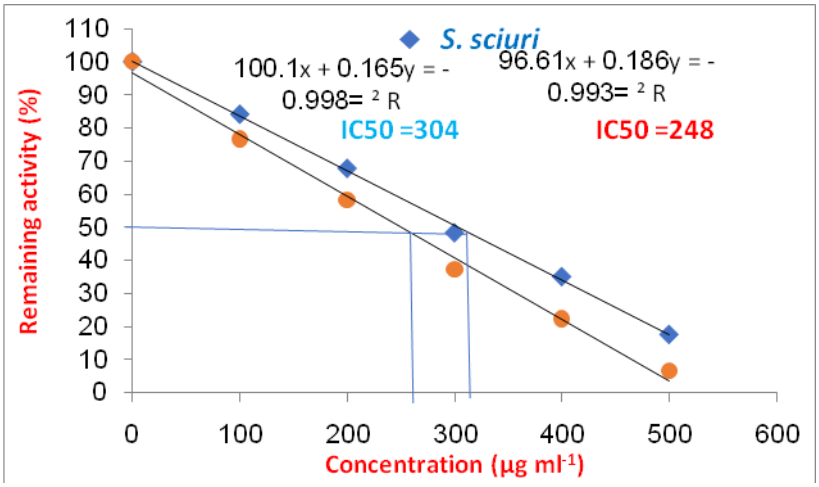

Fig 1: The remaining activity of S. sciuri andK. pneumoniaeβ-lactamase in presence of various concentrations of A. indicaacetone extract.

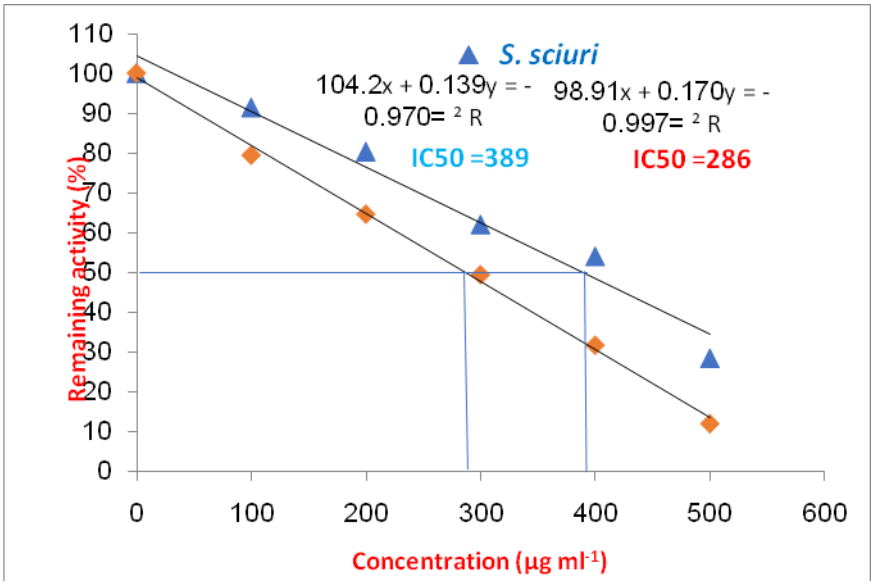

Fig 2: The remaining activity of S. sciuri andK. pneumoniaeβ-lactamase in presence of various concentrations of C. papayaacetone extract.

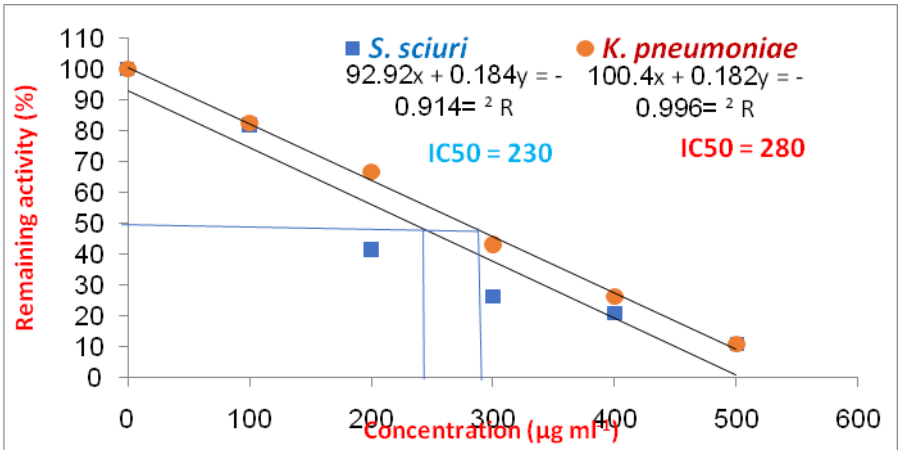

Fig 3: The remaining activity of S. sciuri andK. pneumonia β-lactamase in presence of various concentrations of C. roseusacetone extract.

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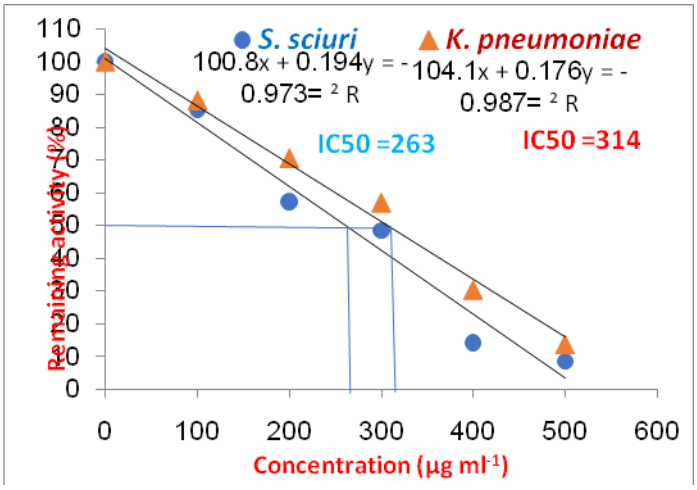

Fig 4: The remaining activity of S. sciuri andK. pneumoniae β-lactamasein presence of various concentrations of C. siliqueacetone extract.

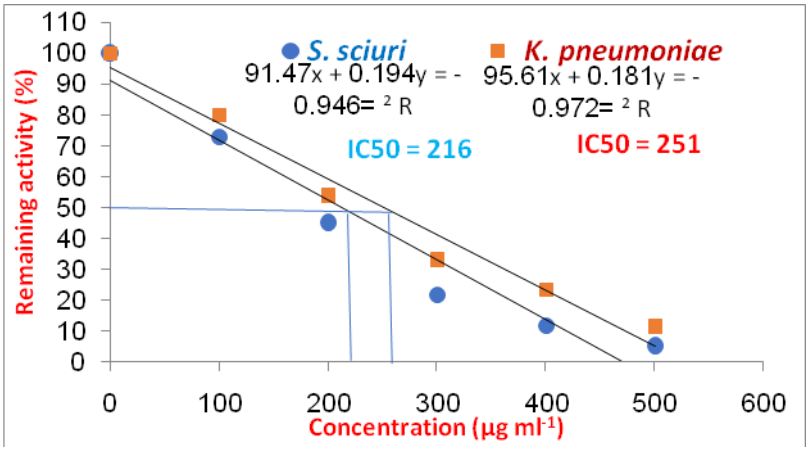

Fig 5: The remaining activity of S. sciuri andK. pneumoniaeβ-lactamas in presence of various concentrations of E. camaldulensisacetone extract.

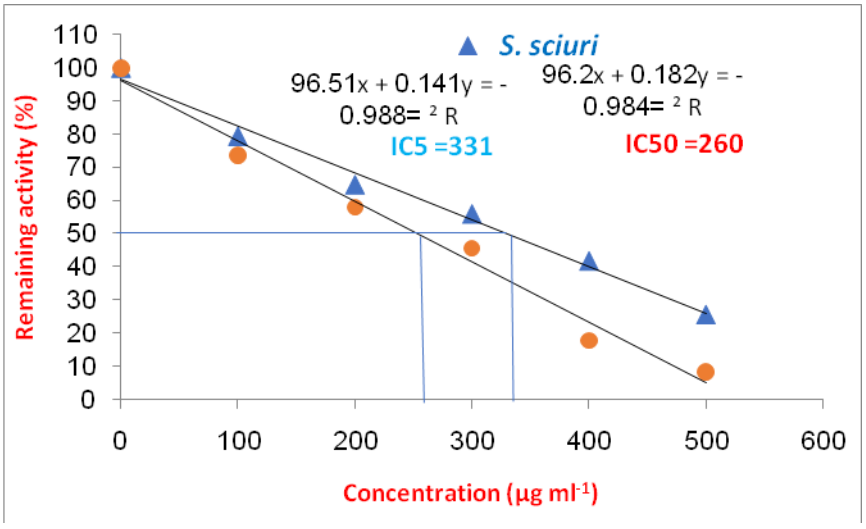

Fig 6: The remaining activity of S. sciuri andK. pneumoniae β-lactamase in presence of various concentrations of F. sycomorusacetone extract.

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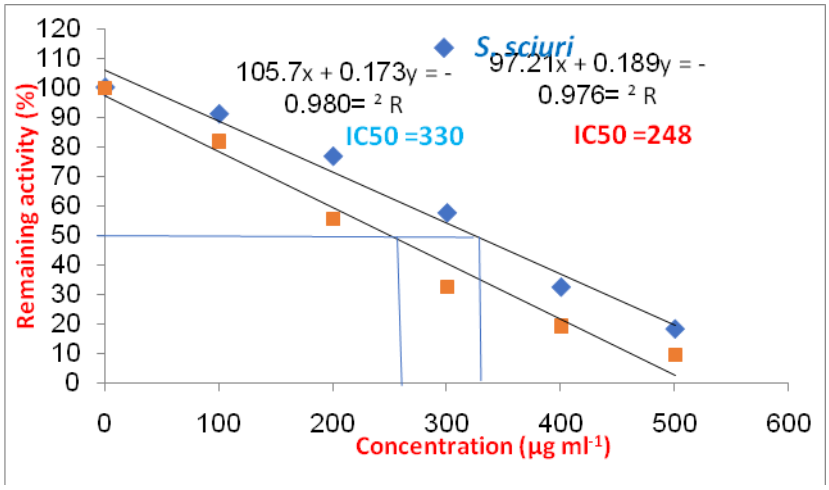

Fig 7: The remaining activity of S. sciuri andK. pneumoniae β-lactamase in presence of various concentrations of M. oleiferaacetone extract.

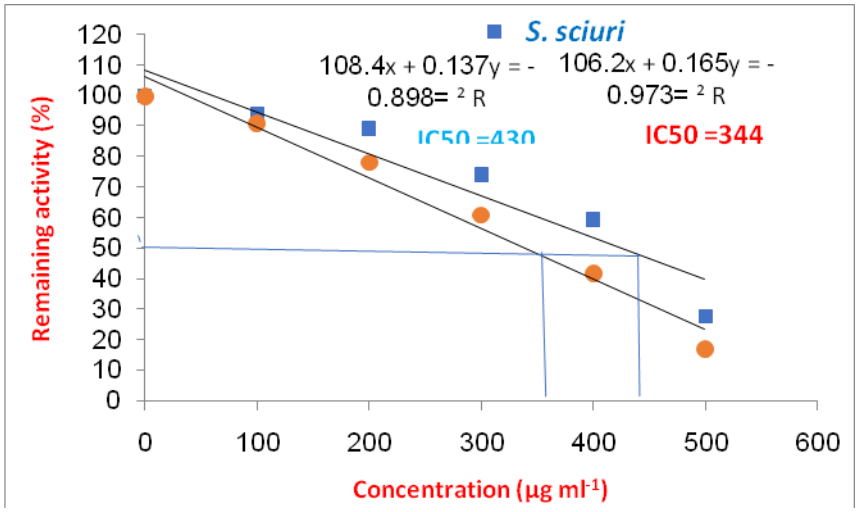

Fig 8: The remaining activity of S. sciuri andK. pneumoniaeβ-lactamase in presence of various concentrations of O. basilicumacetone extract.

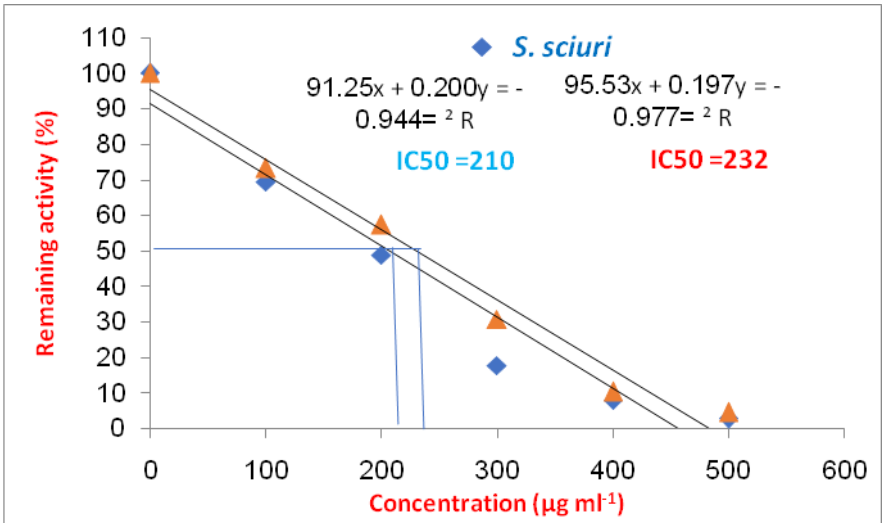

Fig 9: The remaining activity of S. sciuri andK. pneumoniae β-lactamase in presence of various

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concentrations of S. terebinthifoliusacetone extract.

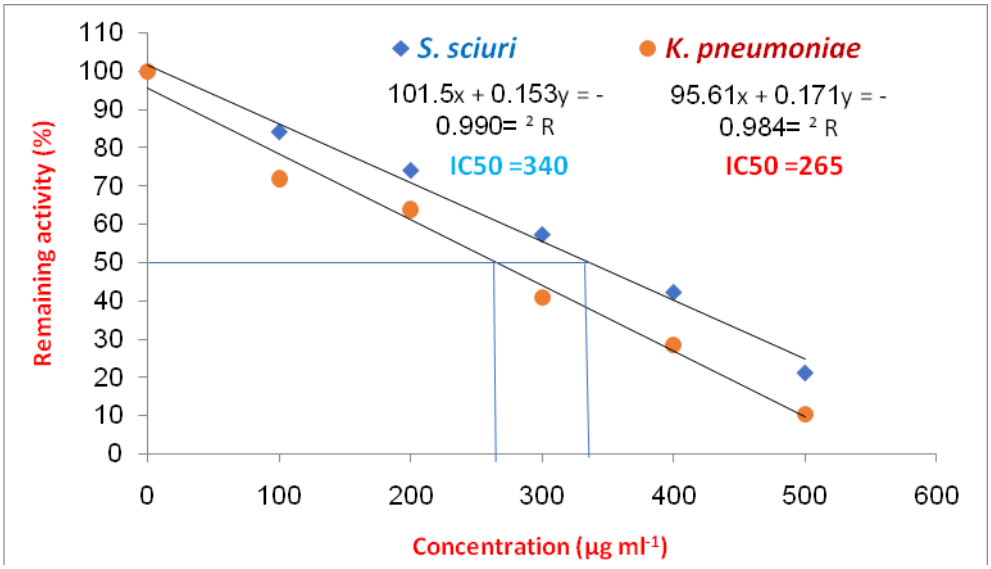

Fig 10: The remaining activity of S. sciuri andK. pneumoniaeβ-lactamase in presence of various concentrations of W. somniferaacetone extract.

Table 1: Summary of IC_{50} of acetone extracts of ten medicinal plants.

Plant extract	IC_{50}	
	S. sciuri β-lactamase	K. pneumoniaeβ-lactamase
A.indica	304	248
C.papaya	389	286
C. roseus	230	280
C.silique	263	314
E.camaldulensis	216	251
F.sycomorus	331	260
M.oleifera	330	248
O.basilicum	430	344
S.terebinthifolius	210	232
W.somnifera	340	265

Determination of Ki for plant extracts at various concentrations

The most effective previous acetone plants extracts including: C. roseus, E. camaldulensis and S. terebinthifolius used to determine Ki value of S. sciuri andK. pneumoniaeβ-lactamase inhibition. The results are shown in Table 2.

For plant extracts of C. roseus, E. camaldulensis and S. terebinthifolius the Ki values for S. sciuri β-lactamase inhibition were 211, 330 and 381 respectively Also, forC. roseus, E. camaldulensis and S. terebinthifolius the Ki for K. pneumoniae β-lactamase inhibition were 77, 221 and 374 respectively (Fig. 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22).

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Table 2: Ki values of the S. sciuri and K. pneumoniaeβ-lactamase inhibition by the three medicinal plant extracts.

plants extracts	Ki values	
	S. sciuri β-lactamase	K. pneumoniae β-lactamase
C. roseus	211	77
E.camaldulensis	330	221
S.terebinthifolius	381	374

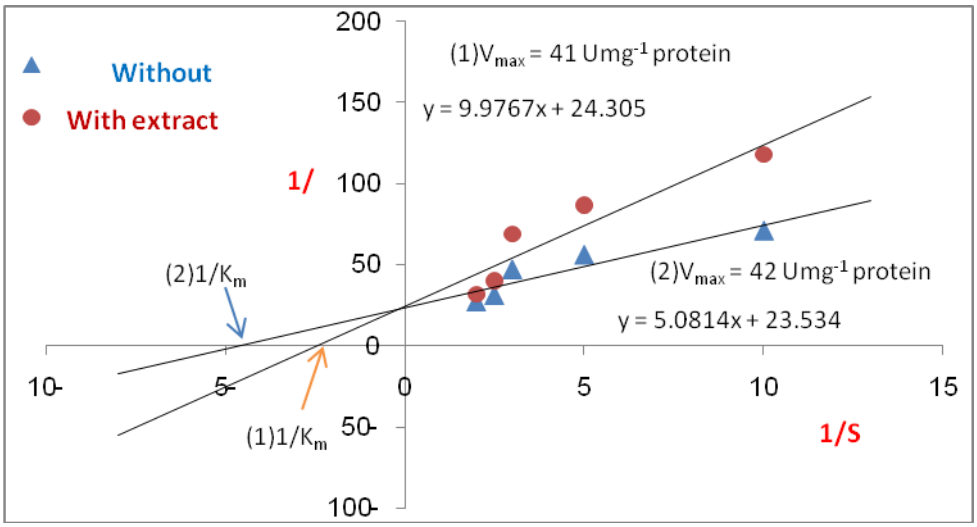

Fig 11: Reciprocal of V against reciprocal of S for β-lactamase from S. sciuri with and without C. roseus acetone extract (Lineweaver-Burk plot).

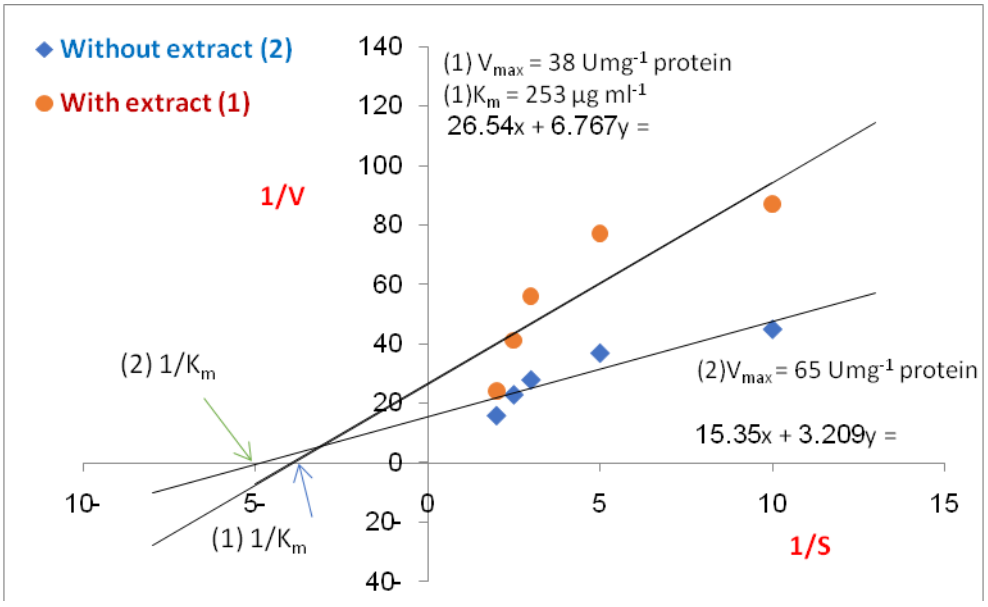

Fig 12: Reciprocal of V against reciprocal of S for β-lactamase from S. sciuri with and without E. camaldulensisacetone extract (Lineweaver-Burk plot).

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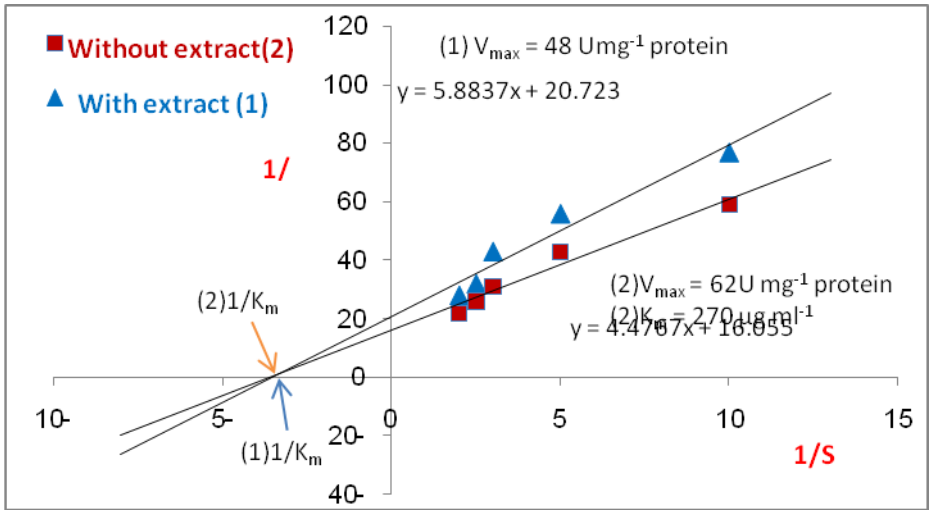

Fig 13: Reciprocal of V against reciprocal of S for β-lactamase from S. sciuri with and without S. terebinthifolius acetone extract (Lineweaver-Burk plot).

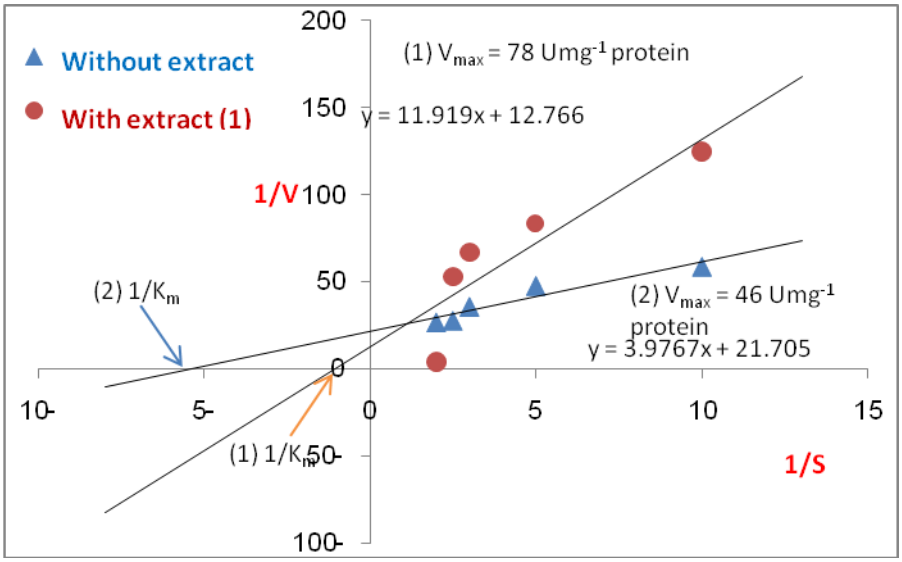

Fig 14: Reciprocal of V against reciprocal of S for β-lactamase from K. pneumoniae with and without C. roseus acetone extract (Lineweaver-Burk plot).

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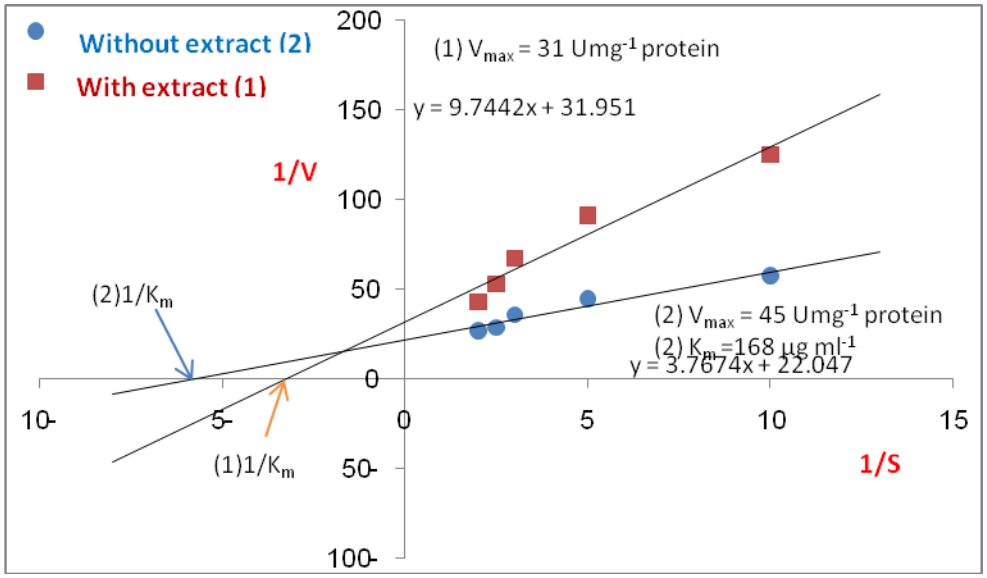

Fig 15: Reciprocal of V against reciprocal of S for β-lactamase from K. pneumoniae with and without E. camaldulensisacetone extra (Lineweaver-Burk plot).

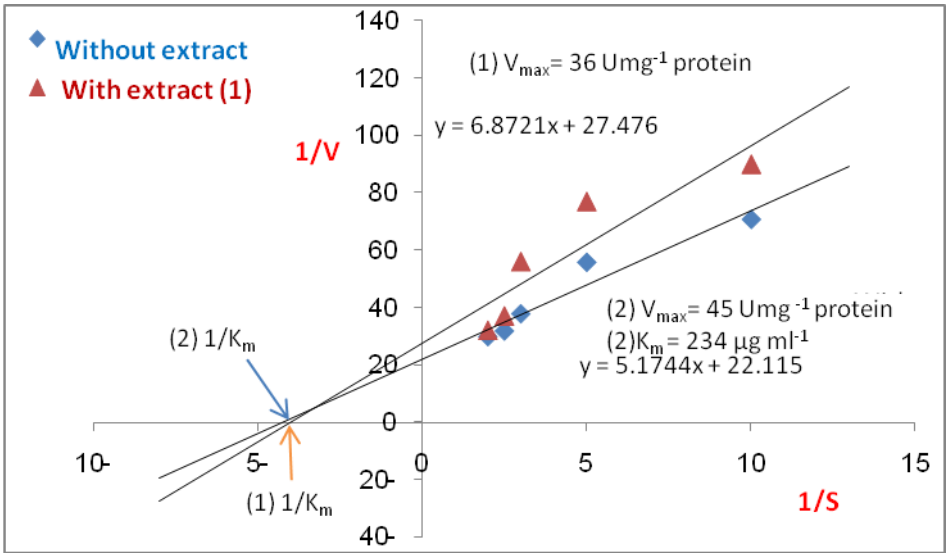

Fig 16: Reciprocal of V against reciprocal of S for β-lactamase from K. pneumoniae with and without S. terebinthifolius acetone extract (Lineweaver-Burk plot).

DISCUSSION

The acetone extracts of ten medicinal plants showed the most promising in vitroβ-lactamase inhibitor activity through the Iodometry method.This may be due to contain the most of tested plants extracts in the present work different phytochemical compounds including flavonoids, alkaloids, tannins, phenols, saponins, steroids, terpenoids and glycosides and may be due tothe better solubility of the active components in organic solvents as acetone [16].

There are little studies about screening for β-lactamase inhibitors from extracts of plants. Attention has been centered intensively on secondary metabolites of plants and the synthesis of compounds [17], and studies their antimicrobial activities [18].

A few reports have referenced the presence of β-lactamase inhibitor effect from specific plants and plant products, while lot of plant biodiversity is yet to be employed [8].

Yang et al. [18]recorded that extracts of 100 conventional Chinese medicines were tested for β-lactamase inhibitors bystarch-iodine agar plate technique, no extracts among these 100 samples demonstrated powerful inhibiting effects against β-lactamase. Of the most inhibitor plants the inhibition rates were found to be 60%.

In investigate for β-lactamase inhibitors from natural sources; Yang et al. [19]reported that the methanol extract of the Fissistigmacavalerieiroots showed an inhibitory effect on β-lactamase. The Inhibitory agent of β-lactamase was recognized as salicylsalicylic.

Aqueous extract ofTerminalia chebula was found to be effective on MBL enzymes which were formed by eight isolates of Acinetobacterbaumannii and eleven isolates ofP.aeruginosa[20].

Al Sahli and Abdulkhair[21]checked six plants cultivating in Saudi Arabia and observed that one extract of Rumexvesicarius L. has antagonistic activity for β-lactamase enzyme. Inhibitory agent of β-lactamase was purified and recognized as clavulanic acid.

Beta-lactamase inhibitory activity of fifteen plants was examined by usinga chromogenic substrate. This plant extracts exhibited significant β-lactamase inhibition [22].

Shaikh et al. [8]investigated the β-lactamase inhibitor activity of 68 extracts from Indian spicesand herbs in vivo and in vitro. Most encouraging results of the β-lactamase inhibitor activity were accomplished from the herbal extracts of brahmi (Bacopamonnieri), baheda (Terminalia ellerica), garlic (Allium sativum), gurmar (Gymnemasylvestre), ginger Zingiberofficinale),pomegranate (Punicagranatum)andsatavar (Asparagus racemosus) seedsand peels.

Furthermore, considerable inhibition of β-lactamase activity was accomplished by the herbal extracts of Calotropisprocera, Allium sativum, Laws oniainermis, Zingiberofficinale and Ocimum sanctumagainst ESBL [23].

The results of our current study may not be in agreement with the results published by the other researchers; these may be probably due to types of selected plants (here plants were chosen on the basis of their conventional indigenous knowledge and ethnobotanical use), themethod of plant extraction, the solvent used in the extraction, the concentrations of extracts, the method used to assess theβ-lactamase inhibitor activity of these plants and the differences in the experimental conditions used for each method.

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